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(71) Applicant *(for all designated States except US)*: ISTITUTO DI RICERCHE DI BIOLOGIA MOLECOLARE [IT/IT]; P. Angeletti S.p.A., Via Pontina Km. 30,600, I–00040 Pomerzia RM (IT).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: PESSI, Antonello [IT/IT]; Via Monte Bianco, 63, I–00141 Roma (IT). STEINKÜHLER, Christian [DE/IT]; Via A. Vigorelli, 7, I–00144 Roma (IT). DE FRANCESCO, Raffaele [IT/IT]; Via Appia Nuova, 133, I–00040 Marino RM (IT).

(74) Agents: DI CERBO, Mario et al.; Società Italiana Brevetti S.p.A., Piazza di Pietra, 39, I–00186 Roma (IT).

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(54) Title: PEPTIDE INHIBITORS OF THE SERINE PROTEASE ACTIVITY ASSOCIATED TO THE NS3 PROTEIN OF HCV, RELEVANT USES AND PROCESS OF PRODUCTION

(57) Abstract

Subject of the invention are peptides capable of inhibiting the serine–protease activity associated to the NS3 protein of HCV virus, their uses and a process for their production comprising the proteolysis of polypeptides containing at least one among the sequences of the NS3/NS4A, NS4A/NS4B, NS4B/NS5A and/or NS5A/NS5B junction sites of the polyprotein of HCV virus.

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PEPTIDE INHIBITORS OF THE SERINE PROTEASE ACTIVITY ASSOCIATED TO THE NS3 PROTEIN OF HCV, RELEVANT USES AND PROCESS OF PRODUCTION.

DESCRIPTION

Field of the invention

The present invention relates to the molecular biology and to the virology of the human hepatitis C virus (HCV). In particular, it relates to the research of molecules that could potentially be adopted in the therapy of the variety of hepatitis consequent to the infection of this virus.

State of the Art

Presently, the method most frequently adopted in art in order to generate molecules with therapeutical potentialities towards viral pathologies, is that of subjecting collections of compounds, containing a large number of single chemical entities of high molecular diversity, to an automatized program to detect the existence of single active agents. Those agents are then subjected to further chemical modifications aimed at improving their therapeutical potential.

In the specific case of HCV, methods allowing in vitro culture and the passage of infective particles in cellular cultures have not been described in art. Moreover, the only animal infection models utilise primates. The high cost of these animal models drastically limits the number of preparations that can be assayed for their antiviral capability. In practice, identification of molecules having a therapeutic potential is at present limited to the identification of molecules capable of interfering with the biological activity of viral proteins somehow expressed outside of the complete viral context. Study of the HCV biology, although heavily hindered by the limitations discussed above, has allowed identification of viral proteins whose biological activity is deemed essential for the viral replication, and whose inhibition is therefore deemed to

have a probable therapeutic usefulness.

The HCV virus is the principal etiologic agent of non-A non-B hepatitis (NANB), whose chronic infection in serum is often a cause of liver cirrhosis and may progress in 20 - 30 years time to hepatocellular carcinoma. Regarding the molecular biology of HCV, as is known, it is a virus with a membrane, containing an encapsidized RNA+ genoma of approximately 9.4 Kb.

The genomic organisation of the HCV virus comprises a structural region, coding for proteins concurring to form the virus structure, and a non-structural region NS, coding for functional proteins (helicase/protease; RNA-dependant RNA polymerase).

Both regions are placed in a single open reading frame (ORF) variable between 9030 and 9099 nucleotides that is translated in a single viral polyprotein, whose length may vary between 3010 and 3033 amino acids, only afterwards, during the viral infection cycle, proteolytically processed in individual genic products. Different molecular biology studies have indicated that the polyprotein ripening is due to different enzymes. In particular, the processing of the nonstructural portion of the HCV polyprotein, comprising the NS2-NS3-NS4A-NS4B-NS5A-NS5B proteins (placed in this order), is due to the activity of two different proteases, on of which is a serine-protease contained inside of the N-terminal region (amino acids 1-181) of the NS3 protein (therefore named NS3 protease), responsible of the cleaving at NS3/NS4A, NS4A/NS4B, NS4B/NS5A and NS5A/NS5B sites (Bartenschlager, R. *Antiviral Chemistry & Chemotherapy* 1997).

NS3 is a 68 KDa protein, in fact showing 2 functional domains, one serine protease domain in the first 200 amino-terminal amino acids and a RNA-dependant ATPase domain at the carboxy- terminus.

Initially the substrate specificity of NS3 protease has been qualitatively investigated using transient

transfection (Kolykhalov, A. et al. *J. Virol.* 1994; Bartenschlager, R., et al. *J. Virol.* 69, 198-205, 1995), in vitro translation (Leinbach, S., et al *Virology* 1994), or intracellular processing of fusion proteins in E.coli (Komoda, Y., et al. *J. Virol.* 1994). More recently, efficient heterologous expression and purification of the enzymatically active protease domain have been described (Shimizu, Y., et al. *J. Virol.* 1996; Steinkühler, C., et al. *J. Biol. Chem.* 1996; Kakiuchi, N., et al. *Biochem. Biophys. Res. Commun.* 1995; Overton, H., et al. *J. Gen. Virol.* 1995; D'Souza, E. D. A., et al. *J. Gen. Virol.* 1995; Suzuki, T., et al. *J. Gen. Virol.* 1995; Shoji, I., et al. *Hepathology* 1996; Mori, A., et al. *FEBS Lett.* 1996; Hong, Z., et al. *Anal. Biochem.* 1996; Steinkühler, C., et al. *J. Virol.* 1996), and optimal conditions for the determination of protease activity have been established (Steinkühler, C., et al. *J. Virol.* 1996; Urbani, A., et al. *J. Biol. Chem.* 1997; Bianchi, E., et al. *Anal. Biochem.* 1996; Taliani, M., et al. *Anal. Biochem.* 1996).

According to what has been described on the virus biology and on the infection and viral replication cycles, it is evident that a substance capable of interfering with the NS3 protein associated proteolytic activity might constitute a new therapeutical agent. In fact, inhibition of this protease activity would entail the stopping of the proteolytic processing of the non-structural region of the HCV polyprotein and would, therefore, hinder viral replication in infected cells.

The development of methods enabling the production of enzymatically active NS3 and of enzymatic activity assay methods allowed the setting-up of research programs of new chemical entities, capable of interfering with the NS3 protease activity. These programs essentially consist of the introduction in the enzymatic activity assays of a large number of single chemical entities in order to determine their specific activity on protease. Compounds

thus defined as active, are then subjected to further chemical modifications, aimed at improving their therapeutic potential. A second commonly adopted approach comprises the rational modification of substrates ligands of the protease, in order to develop compounds, capable of altering or abolishing biological activity, with a high binding affinity.

Summary description of the invention

The subject of the present invention are peptides capable of inhibiting protease activity associated to the HCV NS3 enzyme. They have been identified during studies on NS3 enzyme substrate specificity, due to the identification among products of NS3 proteolytic action on the viral polyprotein, of some peptides capable of acting as inhibitors of the protease itself.

In particular, it was found that proteolysis-derived peptides bearing in the C-terminal portion of their sequence the amino acids naturally occurring in P4, P3, P2 and P1 positions (according to the definition of Schechter, I. and Berger, A., 1967) of the junction sites NS3/NS4A, NS4A/NS4B, NS4B/NS5A, and NS5A/NS5B, exhibit an inhibitory capacity towards the NS3 protease itself. The sequences of the abovementioned four cleaving sites of the NS3 enzyme are listed in table I.

TABLE I: Sequence of the NS3 cleaving sites

Cleaving site	Sequence
NS3/NS4A	D L E V V T **S** T W V
NS4A/NS4B	D E M E E C **A** S H L
NS4B/NS5A	D C S T P C **S** G S W
NS5A/NS5b	E D V V C C **S** M S Y

P_6-P'_4 residues of HCV Bk strain polyprotein cleaving sites. Amino acids in the sequences are indicated with the one-letter code. P_1 and P'_1 are bolded.

Among peptides of viral origin, a particular inhibitory effectiveness was evidenced in the two peptides indicated in the sequence listing as SEQ ID NO:1 and SEQ ID NO: 8, the sequence thereof corresponds

to the P6-P1 residues respectively of sites NS4A/NS4B and NS5A/NS5B.

The fact that products of the enzymatic action are capable of acting as competitive inhibitors of the enzyme responsible of their production is very unusual for a serine protease as NS3, and therefore unexpected, opening new perspectives for the development of more effective drugs against nonA-nonB hepatitis.

Following the characterization of such peptides, it was further assessed that such inhibitory capability can be specifically ascribed to the presence of at least a free acid function in the C-terminal position of such peptides. The amino acids in the other positions of the peptides, although significantly affecting the level of inhibitory capability of the peptides, can not by themselves confer inhibitory properties to the same peptides.

Accordingly, in correspondence to each position have been identified the amino acid or the amino acids increasing the relevant inhibitory capability. Hence further peptides, presenting at their C-terminal position an acid function, have been chemically synthesised, whose amino acidic sequence is partly obtained by viral peptides sequences, characterised in that they show a remarkable increase of inhibitory capacity.

In any case, as the sequence of the peptides of viral origin corresponds to the P6-P1 residues of the viral sites, which they are derived from, the positions occupied by each amino acid residue in all the peptides obtained have been conventionally denominated from P6 to P1, P6 being the the position of the N-terminal end and P1 being the position of the C-terminal end.

In relation to that, and to what will be disclosed hereinafter, subject of the present invention is first of all peptides consisting in six amino acid residues arranged in positions from P6 to P1, P6 being the

position of the N-terminal end and P1 being the position of the C-terminal end, characterized in that the amino acid in the P1 position has at least a free acid function and in that they are capable of inhibiting the protease activity of the HCV virus associated to the NS3 protein.

In particular subject of the invention are:

- the peptides wherein the amino acid in P1 position is a cysteine, an analog or a derivative thereof, and in particular an amino acid selected from the group comprising L-cysteine, D-cysteine, homocysteine, S-methylcysteine, alanine, S-ethylcysteine, threonine, methionine, serine and penicillamine;

- the above mentioned peptides having in P6 position an acid function, in particular selected from the group comprising aspartic acid, succinic acid and acylsulfonamide;

- the above mentioned peptides having in P5 position an acid function, in particular selected from the group comprising aspartic acid, succinic acid, acylsulfonamide;

- the above mentioned peptides having in the P4 position an hydrophobic amino acid, in particular selected from the group comprising 3,3,diphenilalanine, leucine, isoleucine and phenylglicine;

- the above mentioned peptides having in the position P3 an amino acid selected from the group comprising glutamic acid, valine and isoleucine, and in a realization form having in the position P5 an amino acid selected from the group comprising aspartic acid, p-nitrophenylalanine, tyrosine, g-carboxyglutamic acid, D-phenylalanine, D-tyrosine, D-valine, D-isoleucine, D-3,3-diphenylalanine, D-aspartic acid, D-glutamic acid and D-g-carboxyglutamic acid, in another realization form, together with such amino acid in P5 position or not, in the position P1 an amino acid selected from the

group comprising aminobutyric acid, norvaline and valine.

Cases of particular relevance are the one wherein the peptides are capable of inhibiting 50% of the NS3 enzymatic activity at a concentration lower than or equal to 2 μM (IC_{50}), and the one wherein the peptides have in the positions P4, P3, P2 and P1, the amino acids naturally occurring respectively in P4, P3, P2 and P1 positions of one of the junction sites of the HCV virus, said junction sites being selected from the group comprising NS3/NS4A, NS4A/NS4B, NS4B/NS5A, and NS5A/NS5B.

Further subject of the present invention are the peptides obtainable by the proteolysis reaction of polipeptides containing at least one of the junction sites of the polyprotein of said HCV virus, said junction sites being selected from the group consisting of NS3/NS4A, NS4A/NS4B, NS4B/NS5A and NS5A/NS5B junction sites.

Thus, are of particular relevance the case wherein the junction sites consist of decapeptides, containing the amino acids naturally occurring in the positions P4, P3, P2 and P1 of NS3/NS4A, NS4A/NS4B, NS4B/NS5A and NS5A/NS5B junction sites; the case wherein the HCV viruses is selected from the group comprising HCV virus of 1a, 1b, 1c, 2a, 2b, 2c, 2d, 2e, 2f, 3a, 3b, 3c, 3d, 3e, 3f, 4a, 4b, 4c, 4d, 5a, 5a, 6b, 7a, 7b, 7c, 7d, 8a, 8b, 9a, 9b, 9c, 10a and 11a genotype, described as non-limiting examples in Tokita, M. et al J. of Gen. Virol. 1996; and in Myakawa, Y., et al, Molecular Med. Today, 1995, and the case wherein the virus is of H-FDA, H-AP, HCV-1, HCV-J, HCV-BK, HC-J6, HCV-T, HC-J8, HCV-JT and/or HCV-JT' strain described as non-limiting examples in Grakou et al, J. of Virol., 1993.

In a preferred embodiment, the peptides according to the present invention are those having an amino acid sequence selected from the group comprising the

sequences reported in the annexed sequence listing as from SEQ ID NO:1 to SEQ ID NO:69.

A further subject of the present invention is the use of the abovementioned peptides for derivation of binding or inhibition assays of the enzymatic activity of HCV NS3 protease, but above all the utilisation of those peptides for the preparation of drugs for the treatment of non-A non-B hepatitis.

Moreover, of particular relevance is the use that may be done of this peptide inhibitors in the "co-crystallisation" with the enzyme, to obtain structural information on the enzyme active site , thereby facilitating the discovery of new enzymatic activity modulators, of peptidic nature or not.

All peptides as described above can be used to prepare pharmaceutical compositions, characterised in that they comprise beside at least one of the aforedescribed peptides, a pharmaceutically effective carrier, vehicle or auxiliary agent, as well as compositions that likewise comprise at least one of said peptides.

A further subject of the present invention is a process for the production of at least one of the afore mentioned peptide characterized by the step of carrying out the the proteolysis of polypeptides containing at least one among the sequences of the NS3/NS4A, NS4A/NS4B, NS4B/NS5A and/or NS5A/NS5B junction sites of the polyprotein of HCV virus.

In particular, cases wherein the proteolysis reaction is operated by NS3 protease of the HCV virus are considered wherein HCV displays a genotype 1a, 1b, 1c, 2a, 2b, 2c, 2d, 2e, 2f, 3a, 3b, 3c, 3d, 3e, 3f, 4a, 4b, 4c, 4d, 5a, 5a, 6b, 7a, 7b, 7c, 7d, 8a, 8b, 9a, 9b, 9c, 10a and/or 11a, described as non-limiting examples in Tokita, M. et al J. of Gen. Virol. 1996; and in Myakawa, Y., et al, Molecular Med. Today, 1995, and the case wherein the virus is of H-FDA, H-AP, HCV-1, HCV-J,

HCV-BK, HC-J6, HCV-T, HC-J8, HCV-JT and/or HCV-JT' strain described as non-limiting examples in Grakou et al, J. of Virol., 1993.

Another case of particular relevance is the one wherein the junction sites, contained in the NS3 polypeptide substrate, consist of decapeptides, containing the amino acids naturally occurring in P4, P3, P2 and P1 positions of the same junction sites themselves.

The invention will be better understood with the aid of the annexed figures.

Brief description of the drawings

Figure 1 shows the reaction kinetics of the NS4A/NS4B substrate cleaving catalysed by NS3 protease.

Figure 2 shows the determination of the IC_{50} of peptide SEQ ID NO:1 by displacement of the fluorescent marker derived from peptide SEQ ID NO: 69. In fig. 2a the intensity decrease of the fluorescence spectrum of the NS3 protease-peptide complex SEQ ID NO: 69 is plotted against the increasing concentration of the peptide SEQ ID NO: 1. In fig. 2b the variation of intensity of the fluorescence spectrum at 520nm is plotted against the peptide SEQ ID NO: 1 concentration for the IC_{50} assessment.

Detailed description of the invention

The subject of the present invention are peptides having a relevant inhibitory capacity towards of the NS3-associated protease activity, some of which correspond to those of viral origin, others thereby obtained by modifications of one or more amino acid residues.

In table II are particularly reported, as a non-limiting example, codes and features of 69 peptide inhibitors obtained from the study on NS3 enzyme substrate specificity, and the concentration in μM of compound is indicated, whereto 50% inhibition of NS3 enzymatic activity (IC_{50}) is obtained, as a reference

parameter for the assessment of the higher or lower efficiency of inhibitory capacity of the single peptides.

TABLE II: Summary list of sequences of peptide inhibitors according to the invention

SEQ ID	Amino acid sequence	IC_{50}
NO:1	Asp Glu Met Glu Glu Cys	1.0
NO:2	Asp Glu Met Glu Glu (D)Cys	4.0
NO:3	Asp Glu Met Glu Glu Abu	5.8
NO:4	Asp Glu Met Glu Glu Ser	41
NO:5	Asp Glu Met Glu Glu Gly	62
NO:6	Met Glu Glu Cys	150
NO:7	Glu Met Glu Glu Cys	21
NO:8	Glu Asp Val Val Cys Cys	5.3
NO:9	Glu Asp Val Val Abu Cys	2.8
NO:10	Asp Glu Val Val Cys Cys	2.1
NO:11	Glu Asp Val Val Gly Cys	20
NO:12	Asp Glu Met Glu Glu Alg	12
NO:13	Glu Asp Val Val MeGly Cys	21
NO:14	Asp Glu Met Glu Glu CysN	30%@64μM
NO:15	Glu Asp Val MeVal Abu Cys	230
NO:16	Glu Asp MeVal Val Abu Cys	1,3
NO:17	Asp Glu Met Glu Glu Cys(ol)	130
NO:18	GluS Met Glu Glu Cys	1.3
NO:19	MetS Glu Glu Cys	77
NO:20	AsGlu Met Glu Glu Cys	0.6
NO:21	Asp Glu Met Glu Glu VGly	38
NO:22	Asp Glu Met Glu Leu Cys	1.1
NO:23	Asp Glu Met Glu Cha Cys	0.3
NO:24	Asp Glu Met Glu Nap Cys	0.8
NO:25	AspS Val Val Abu Cys	4.6
NO:26	Glu Asp Val Val Abu (D)Cys	194
NO:27	Asp Glu Met Glu Glu Cys(Me)	16.7
NO:28	Asp Glu Val Glu Cha Cys	0.33
NO:29	Asp Glu Ile Glu Cha Cys	0.12

NO:30	Asp Glu Tyr Glu Cha Cys	0.24
NO:31	Asp Glu Phe Glu Cha Cys	0.42
NO:32	Asp Glu Leu Glu Cha Cys	0.12
NO:33	Asp Glu Cha Glu Cha Cys	0.14
NO:34	Asp Glu Nle Glu Cha Cys	0.22
NO:35	Asp Glu Dif Glu Cha Cys	0.05
NO:36	Asp Glu Tha Glu Cha Cys	0.87
NO:37	Asp Glu FCI Glu Cha Cys	0.3
NO:38	Asp Glu Phg Glu Cha Cys	0.12
NO:39	Asp Glu Dif Glu Cha (D)Cys	3.4
NO:40	Asp Glu Met Glu Glu bAla	20%@200μM
NO:41	Asp Glu Met Glu Glu CysAs	4.0
NO:42	Glu Dif Glu Cha Cys	1.4
NO:43	Dif Glu Cha Cys	30
NO:44	Asp Glu Leu Val Cha Cys	0.08
NO:45	Asp Glu Leu Ile Cha Cys	0.06
NO:46	Asp MeGlu Leu Glu Cha Cys	1.0
NO:47	Asp Glu Dif Glu Cha ΔAla	7.1
NO:48	Asp Glu Met Glu Glu Cpc	9.0
NO:49	Asp Glu Dif Ile Cha	46
NO:50	Glu Dif Ile Cha Cys	2.5
NO:51	Dif Ile Cha Cys	100
NO:52	Asp Glu Met Glu Glu CnAla	19
NO:53	Asp Glu Dif Ile Cha Cys	0.06
NO:54	Asp Glu Leu Glu Cha Abu	1.6
NO:55	Asp Glu Leu Glu Cha Val	4.0
NO:56	Asp Glu Leu Glu Cha Nva	1.3
NO:57	Asp Asp Leu Glu Cha Cys	0.290
NO:58	Asp Fno Leu Glu Cha Cys	0.240
NO:59	Asp Tyr Leu Glu Cha Cys	0.135
NO:60	Asp Gla Leu Glu Cha Cys	0.055
NO:61	Asp (D)Phe Leu Glu Cha Cys	0.820
NO:62	Asp (D)Tyr Leu Glu Cha Cys	0.680
NO:63	Asp (D)Val Leu Glu Cha Cys	0.470

NO:64	Asp (D)Ile Leu Glu Cha Cys	0.330
NO:65	Asp (D)Dif Leu Glu Cha Cys	0.276
NO:66	Asp (D)Asp Leu Glu Cha Cys	0.122
NO:67	Asp (D)Glu Leu Glu Cha Cys	0.045
NO:68	Asp (D)Gla Leu Ile Cha Cys	0.0015
NO:69	Asp Glu Dpr(N-b-Dns)Glu Cha Cys	0.4

Abu = 2-aminobutyric acid

Alg = allylglycine

AsGlu = Glu presenting an acylsulfonamide in N-terminus position

AspS = Asp whereto a succinil group is bound

bAla = beta-alanine

Cha = beta-cyclohexylalanine

CnAla = cyanoalanine

Cpc = 1-amino-1-cyclopentan-carboxylic acid

CysAs = Cys presenting in C-terminal position an acylsulfonamide

Cys(Me)= S-methylcysteine

Cys(ol) = cysteinol

CysN = cysteamine

Dpr = b-diaminopropionic acid

ΔAla= dehydroalanine

Dif = 3,3-diphenylalanine

Dns = Dansyl (5-Dimethylamino-1-naftalensulfonyl)

FCl = 4-clorophenylalanine

Fno = 4-nitrophenylalanine

Gla = g-carboxyglutamic acid

GluS = Glu whereto a succinyl group is bound

MetS = Met whereto a succinyl group is bound

MeGlu = N-methyl-glutamic acid

MeGly = methyl-glycine

MeVal = methyl-Val

Nap = naphtylalanine

Nle = norleucine

Nva = norvaline

Phg = phenylglycine

Tha = 2-tienylalanine

VGly = Vinylglycine

Of the peptides listed in Table II, as already said, two (SEQ ID NOS:1 and 8) are produced directly from the cleaving of the NS3 itself, respectively on the 4A/4B site (SEQ ID NO:1) and on the 5A/5B site (SEQ ID NO:8) of the viral polyprotein. This inhibition can be evidenced studying the time-dependence of the proteolytic cleaving reaction, mediated by the NS3 enzymatic activity, of a substrate corresponding to site 4A/4B (see Table I). Figure 1 shows that the enzymatic conversion of this peptide in its cleaving products decreases over time. Using methods known in the art it is possible to estimate that this NS3 protease activity decrease is consistent with the forming, during the proteolytic cleaving reaction, of a product that inhibits the enzyme with a Ki constant, defined as the dissociation constant of the enzyme-inhibitor complex, of 600 nM. Comparing values indicated in the above table, a remarkable increase is clearly evident of the inhibitory capacity of the most part of the synthetic peptides, as compared to the capacity related to peptides of viral origin.

Results are reported in detail hereinafter, with reference to substitutions of amino acids in P_1-P_6 positions of viral peptides sequence (SEQ ID NO:1, SEQ ID NO:8).

P_1 Residue

The substitution of a cysteine in the P_1 position with a cysteamine, as in SEQ ID NO:14, or its reduction to an alcohol as in SEQ ID NO:17 (both belonging to the series derived from SEQ ID NO:1) entails a decrease of the inhibitory capacity of a >100-fold factor. The carboxylic group of the cysteine was substituted by an acylsulfonamide group in the peptide represented by SEQ ID NO:41.

P_5 and P_6 residues

With reference to both the series derived from SEQ ID NO:1 (IC_{50} = 1.0 μM) and from SEQ ID NO:8 (IC_{50} = 5.3 μM), the presence of an acid seems to be important, in P_5 as well as in P6. Actually, if P_6 deletion from SEQ ID NO:1 causes a significant decrease of the inhibitory activity(SEQ ID NO:7, IC_{50} = 21 μM), the deletion of both residues causes a 100-fold decrease (SEQ ID NO:6, IC_{50} = 150 μM).

This result is confirmed also when operating the same modifications in more potent analogs like SEQ ID NO:35 (IC_{50} = 0.055 μM) and SEQ ID NO:53 (IC_{50} = 0.063 μM). In the first case SEQ ID NO:42 (IC_{50} = 1.4 μM) and SEQ ID NO:43 (IC_{50} = 30 μM) are obtained; in the second case, SEQ ID NO:50 (IC_{50} = 2.5 μM) and SEQ ID NO:51 which yields 50% inhibition at a 100 μM concentration.

However, aspartic acid in P6 of SEQ ID NO:1 can be replaced with a simple carboxylic acid, like succinic acid (with loss of the acetylaminic moiety), or with an acylsulfonamide without observing a significant decrease of the inhibitory capacity (compare SEQ ID NO:18, IC_{50} = 1.3 μM e SEQ ID NO:20, IC_{50} = 0.6 μM). This has also been verified for the series derived from SEQ ID NO:8, with SEQ ID NO:25 (IC_{50} = 2.8 μM) active as SEQ ID NO:9 (IC_{50} = 2.8 μM).

Lastly, the two acids in P_5 and P_6 SEQ ID NO:1 are interchangeable (compare SEQ ID NO:8, IC_{50} = 5.3 μM, with SEQ ID NO:10, IC_{50} = 2.1 μM).

P_2 substitutions

The effect of the P2 substitutions was studied in both the series derived from the original viral peptides. As for the series derived from SEQ ID NO:8 it was observed that while the substitution of the P2 cysteine with aminobutyric acid as in SEQ ID NO:9 (IC_{50} = 2.8 μM) is tolerated, Gly in the same position results in a peptide 10-fold less active (SEQ ID NO:11, IC_{50} = 20 μM).

A more dramatic effect is observed in the SEQ ID NO:1 derived series, where substitution of the glutamic acid in P_2 with an hydrophobic residue maintains or even improves the inhibitory activity (SEQ ID NO:22, IC_{50} = 1.1 μM; SEQ ID NO:24 IC_{50} = 0.8 μM; SEQ ID NO:23, IC_{50} = 0.3 μM).

P_4 substitutions

SEQ ID NO:23 was taken as a starting point to optimise the P_4 position. This was realised synthesising a series of analogs with the general structure of the starting sequence, presenting modifications only on the P_4 position.

Results showed that the P_4 position has a strong preference for hydrophobic amino acids, both with aliphatic and aromatic side chains, the best residue being 3,3-diphenylalanine (SEQ ID NO:35, IC_{50} = 0.055 μM), followed by leucine (SEQ ID NO:32, IC_{50} = 0.118 μM), isoleucine (SEQ ID NO:29, IC_{50} = 0.122 μM) and phenylglycine (SEQ ID NO:38, IC_{50} = 0.120 μM).

P_3 substitutions

SEQ ID NO:32 was taken in turn as a starting point to optimise the P_3 position. As for the P_4 position, the result was obtained systematically by synthesising a series of analogs that, though presenting the same structure of the SEQ ID NO:32, were modified in P_3 position only.

Only two residues yielded a potency comparable with the glutamic acid in P3 of the SEQ ID NO:32, i.e. valine and isoleucine in P3.

P5 substitutions

SEQ ID NO:32 was again taken as a starting point to optimise P_3 position. As for P_3 and P_4 positions, the result was obtained systematically by the synthesis of a series of analogs that, though presenting the same structure of the SEQ ID NO:32, were modified in P5 position only. The most notable L-amino acids in this position are P_5 = aspartic acid (SEQ ID NO:57, IC_{50} =

0.290 μM), P_5 = p-nitrophenylalanine (SEQ ID NO:58, IC_{50} = 0.240 μM), P_5 = tyrosine, (SEQ ID NO:59, IC_{50} = 0.135 μM) e P_5 = g-carboxyglutamic acid (SEQ ID NO:60, IC_{50} = 0.055 μM). Also amino acids with a D chirality are well tolerated in this position, and in fact the two more potent compounds show this chirality: P_5 = D-phenylalanine (SEQ ID NO:61, IC_{50} = 0.820 μM), P_5 = D-tyrosine (SEQ ID NO:62, IC_{50} = 0.680 μM), P_5 = D-valine (SEQ ID NO:63, IC_{50} = 0.470 μM), P_5 = D-isoleucine (SEQ ID NO:64, IC_{50} = 0.330 μM), P_5 = D-3,3-diphenylalanine (SEQ ID NO:65, IC_{50} = 0.276 μM), P_5 = D-aspartic acid (SEQ ID NO:66, IC_{50} = 0.122 μM), P_5 = D-glutamic acid (SEQ ID NO:67, IC_{50} = 0.045 μM) and P_5 = D-g-carboxyglutamic acid (SEQ ID NO:68, IC_{50} = 0.0015 μM).

P1 substitutions

The effects of the P_1 residue in the SEQ ID NO:1 derived inhibitor series also parallels the trend observed for the substrate. In order of decreasing IC_{50} the residues are: cysteine(SEQ ID NO:1, IC_{50} = 1 μM), aminobutyric acid (SEQ ID NO:3, IC_{50} = 5.8 μM), 1-amino-1-cyclopentancarboxylic acid(SEQ ID NO:48, IC_{50} = 9 μM), allylglycine (SEQ ID NO:12, IC_{50} = 12 μM), S-methyl-cysteine(SEQ ID NO:27, IC_{50} = 17 μM), cyanoalanine (SEQ ID NO:52, IC_{50} = 19 μM), vinylglycine (SEQ ID NO:21, IC_{50} = 38 μM), serine (SEQ ID NO:4, IC_{50} = 41 μM), glycine (SEQ ID NO:5, IC_{50} = 62 μM), β-alanine (SEQ ID NO:40, 20% inhibition at a 200 μM concentration).

The chirality of the P_1 cysteine must be L- in the SEQ ID NO:8, since inversion of chirality yields a 70-fold decrease in activity (SEQ ID NO:26, IC_{50} = 194 μM).

Likewise, the D-cysteine for L-cysteine exchange is highly detrimental of the inhibitory capacity in the more potent analogs modified in P_2 and P_4 positions (compare SEQ ID NO:35, IC_{50} = 0.05 μM and SEQ ID NO:39, IC_{50} = 3.4 μM).

L-cysteine cannot be exchanged with D-cysteine in SEQ ID NO: 8 (compare SEQ ID NO:9, IC_{50} = 2.8 μM and SEQ

ID NO:26, IC_{50} = 194 μM).

Further analysis were carried out using as a basis the more potent analog SEQ ID NO:32 (IC_{50} = 118 nM). These analysis confirmed that cysteine substitution causes anyhow a 10-fold decrease in inhibitory activity; the best substitute is aminobutyric acid (SEQ ID NO:54, IC_{50} = 1.6 μM) together with norvaline (SEQ ID NO:56, IC_{50} = 1.3 μM), followed by valine (SEQ ID NO:55, IC_{50} = 4.0 μM).

Deletion of the P_1 residue in SEQ ID NO:49 yields a >700-fold decrease in activity.

N-methylated Peptidomimetics derived from SEQ ID NO:1 and SEQ ID NO:8

As already said, beside having examined the effects of the substitution of the amino acid residues in positions P_1 and P_6 of the original viral peptides, we have systematically examined also the effects of N-methylation of the bond peptide in a series of analogs always derived from sequences SEQ ID NO:1 and SEQ ID NO:8.

So far, only a general description has been given of the present invention. With the aid of the following examples, a more detailed description will now be given of specific embodiments thereof, with the purpose of giving a clearer understanding of objects, features, advantages and methods of application of the invention. For the sake of simplicity, in the examples the amino acid residues are also indicated with the one-letter code.

Example 1

Enzyme preparation

Escherichia coli BL21(DE3) cells were transformed with a plasmid containing the cDNA coding for the serine protease domain of the HCV BK strain NS3 protein (amino acids 1-180) under the control of bacteriophage T7 gene 10 promoter. The protease domain was purified as previously described (Steinkühler, C. et al., *J. Biol.*

Chem. 1996). The enzyme was homogenous as assessed with electrophoresis on polyacrylamide gel in presence of sodium dodecyl sulphate (SDS-PAGE) using as detector the silver stain, and over 95% pure as assessed from reversed phase HPLC carried out using a 4.6 x 250 mm Vydac C4 column. Enzyme preparations were routinely checked by mass spectrometry done on HPLC purified samples, using a Perkin Elmer API 100 instrument, and N-terminal sequence analysis carried out using Edman degradation on an Applied Biosystems model 470A gas-phase sequencer. Both techniques indicated that in more than 90% of the enzyme molecules the N-terminus methionine and alanine have been removed, yielding an enzyme starting with proline in position 2. Enzyme stocks were quantitated by quantitative analysis of the amino acidic content, shock-frozen in liquid nitrogen and kept in aliquots at -80°C until use. Control experiments have proved that this freezing procedure does not interfere with the specific activity of the enzyme.

Peptide synthesis

Peptide synthesis was performed by Fmoc chemistry(Phluorenhylmethyl-oxycarbonyl)/t-Bu (*tert*-buthyl) chemistry , essentially as described in Atherton and Sheppard.(1989). Peptides were assembled on a Novasyn® TGA (Novabiochem) resin and cleaved off the polymer at the end of the synthesis with TFA 88%, phenol 5%, triisopropylsilane 2%, water 5% (Sole, N. A. and Barany, G. *J. Org. Chem.* 1992).

Crude peptides were purified by reversed-phase HPLC on a Nucleosyl C18, 250 x 21 mm, 100 Å, 7 µm using water, 0.1% TFA and acetonitrile 0.1% TFA as eluents. Analytical HPLC was performed on Ultrasphere C18, 250 x 4.6 mm, 80 Å, 5 µm (Beckman). Purified peptides were characterised by mass spectrometry, [^{1}H]-NMR and amino acid analysis.

HPLC protease activity assay

Concentration on stock solutions of peptides, prepared in DMSO or in buffered aqueous solution and kept at -80°C until use, was determined by quantitative amino acid analysis performed on azeotropic HCl-hydrolysed samples. If not differently specified, cleaving assay was performed in 57 µl 50 mM Tris pH 7.5, 2% CHAPS, 50% glycerol, 10 mM in DTT (buffer A), to which 3 µl of the substrate peptide Ac-DEMEECASHLPYK(Ac)-NH_2 were additioned. As protease co-factor a peptide spanning the central hydrophobic core (residues 21-34) was used of the NS4A protein, with a three-lysine tag at the N-terminus to increase solubility (Bianchi, E. et al., *Biochemistry* 1997), Pep4AK (KKKGSVVIVGRIILSGR-NH_2). PeP4AK was pre-incubated for 10 minutes with 10-50 nM protease prior to the addition of the substrate. Incubation time was chosen in order to obtain a substrate conversion of less than 7%. The reaction was stopped by addition of 40 µl 1% TFA, and the extent of substrate cleaving was determined by HPLC using a Merck-Hitachi chromatograph equipped with an autosampler. 80 µl of sample was injected on a Lichrospher C-18 reversed phase cartridge column (4 x 75 mm, 5 µm, Merck) and fragments were separated using a 10-40% acetonitrile gradient at 5%/min using a flow rate of 2.5 ml/min. Peak detection was accomplished by monitoring both absorbance at 220 nm and fluorescence of the tyrosine residue (λ_{ex} = 260 nm, λ_{em} = 305 nm). Cleaving products were quantitated by integration of chromatograms with respect to appropriate standards. Initial rates of cleaving were determined on samples characterized by a substrate conversion rate of less than 7%. Kinetic parameters were calculated from the initial rates as a function of substrate concentration with the help of Kaleidograph® software, assuming Michaelis-Menten kinetics.

Microplate protease activity assay

The HCV-protease (J strain) was stored until use at

-80°C in 250 mM NaCl, phosphate buffer pH 6.5, 50% glycerol, 0.1% CHAPS; PeP4AK was stored at -80°C in DMSO; the tritiated substrate Ac-DEMEECASHLPYK (^{3}H-Ac)-NH_2 and the corresponding cold substrate Ac-DEMEECASHLPYK(Ac)-NH_2 were stored at -80°C in DMSO/DTT.

The assay was run in Costar polypropilene 96-well plates. The composition of the reaction mixture was as follows (100 µl):

Glycerol	15%
DTT	30 mM
Hepes pH 7,5	50 mM
Triton X-100	0.05%
Protease	10 nM
hot + cold substrate	5 µM (300.000 cpm)
PeP4AK	15 µM

The reaction mixture was diluted in DMSO (final concentration 10% DMSO)

PeP4AK was pre-incubated with protease for 5 min prior to addition of substrate mix. In these conditions, the substrate Km was 7±2 µM. Plates were shaken for 30 minutes at room temperature, then a ionic exchange resin (100 µl of 20% Fractogel TSK-DEAE® 650S, Merck) was added to capture unprocessed substrate and plates shaken for another 10 minutes. After allowing the resin to settle by gravity, 30 µl of the reaction mix were transferred in a 96-well plate (Picoplate, Packard), admixed with 250 µl of scintillation cocktail Microscint 40, and the radioactivity measured in a scintillation Packard Top Count β-counter.

Example 2

Competition assay based on product inhibitors

The property of peptides, derived from the cleaving of the NS3 protease substrates, of binding to the active site of the enzyme, is exploitable for the development of competition assays wherein an inhibitor peptide specifically marked is replaced by another molecule binding to the same site. This technology is exploitable

for the identification of NS3 protease competitive inhibitors. The marking of the inhibitor peptide can be obtained with the introduction of functionalities chemical, radioactive, fluorescent, luminescent or coloured using techniques known in art. For instance, introduction techniques of ^{125}I atoms in peptides containing tyrosine residues are known. It is also possible to synthesise peptides binding the active site of NS3 protease using amino acid residues marked with radioactive isotopes like ^{3}H, ^{14}C or ^{35}S. In art, even chemical modification techniques are known of peptides that can be adopted to introduce a radioactive marker in a peptide using reagents containing radioisotopes. For example, it is possible to mark with ^{3}H a peptide sequence containing primary aminic groups by reaction of said groups with acetic anhydride containing ^{3}H.

Peptides binding NS3 active site containing radioisotopes can be adopted to find other compounds binding the same site using techniques known in art. For instance, a peptide having a sequence that binds to NS3 protease active site marked using the abovedescribed techniques can be added to a buffered solution containing NS3 protease or NS3 protease and its cofactor NS4A, or peptides deriving from the sequence of this cofactor. The protease bound to the peptide can be isolated using filtration techniques, chromatographic resins bonding, or precipitation using saline solutions or organic reagents. The amount of marked peptide can easily be determined using detecting techniques of the radioactive decay process as scintillation. In this process, the addition of a substance capable of binding to the NS3 active site prior to protease isolation using said techniques, entails the displacing of the marked peptide and therefore a reduction in the emission of the radioactive decay products.

It is also possible to introduce in a peptide having a sequence binding to the NS3 protease active

site a chemical functionality with fluorescent properties. It is known in art that the spectroscopic properties of some chemical functionalities undergo alterations depending on the physic-chemical conditions wherein the spectroscopic propterties are determined. These conditions comprise pH, ionic strength, dielectric constant and the specific solvent wherein spectroscopic measurements are carried out. In particular, it is known that some molecules, once bound to proteins undergo spectroscopically detectable changes. Some of these molecules are described in "Handbook of Fluorescent Probes and Research Chemicals" and are commercially available. Others are obtainable with chemical modifications of molecules of known spectroscopic properties, capable of placing them in the context of a peptide binding to the NS3 protease active site. Examples of chemical functionalities that can be used with this aim are: fluorescein, mansyl, coumarin, rhodamine and dansyl. Particularly, dansyl was proved capable of an interaction with tryptophan residues of resonance energy transfer. In this process, tryptophan is excited at a wavelength of between 280 and 295 nm and transfers its excitation energy to the dansyl molecule, that in turn emits energy at a wavelength of between 510 and 540 nm. The phenomenon of resonance energy transfer decays with the sixth power of the distance and is operative at distances of between 10 and 100 Å, making it extremely sensitive to determine the bond between two molecules.

The molecule in SEQ ID NO:69 is a hesapeptide derived by the optimization of the sequence of a NS3 protease cleaving product, SEQ ID NO:23, wherein the methionine residue was replaced with an 2,3-diaminopropionic acid residue, derivatized on P3 amino group with the dansyl group.

It has been proved that the molecule in SEQ ID NO:69 binds to the NS3 protease active site with a

Ki=200 nM. Its bond with protease can be determined with fluorescence spectroscopy. In particular, it is possible to excite the functionality of the dansyl present in the molecule both directly, using light with a 335 nm wavelength or, exploiting the presence of two tryptophans in the NS3 protease, indirectly using the aforementioned phenomenon of resonance energy transfer between NS3 tryptophans and the molecule SEQ ID NO:69 bound to the enzyme. In both cases the bond is directly observable by virtue of the different spectroscopic properties of free and bound molecules. However, utilisation of the resonance energy transfer phenomen is to be preferred as more sensitive.

The SEQ ID NO:69 molecule can be utilised to determine the binding of other molecules to NS3 protease active site, capable therefore of displacing it from the interaction with the enzyme. A typical experiment is shown in Fig. 2. To a buffered solution containing NS3 protease 200 nM complexed with Pep4AK were added SEQ ID NO:69 200 nM. The bond of the two molecules was measured exciting NS3 tryptophans at a 280 nm wavelength and recording emission spectrum around 520 nm. Addition of the NS3 protease competitive inhibitor SEQ ID NO:1 causes a deplacement of SEQ ID NO:69 from NS3 active site and a concomitant reduction of the phenomenon of fluorescence energy transfer. From this experiment it is possible to determine an IC_{50} value for SEQ ID NO:1 of 1 μM, that is the same value found assaying the effect of this molecule on the NS3 protease activity.

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ABBREVIATIONS AND SYMBOLS USED IN THE TEXT

CHAPS = 3-[(3-colamidopropyl)-dimethyl-ammonium]-1-propan-sulfonate;

HPLC = high-performance liquid chromatography;

TFA = Trifluoroacetic acid;

ORF = Open Reading Frame;

NMR = Nuclear Magnetic Resonance

DMSO = Dimethylsulfoxide

DTT = Ditiotreithol

CLAIMS

1. Peptides consisting in six amino acid residues arranged in positions from P6 to P1, P6 being the position of the N-terminal end and P1 being the position of the C-terminal end, characterized in that the amino acid in the P1 position has at least a free acid function and in that they are capable of inhibiting the protease activity of the HCV virus associated to the NS3 protein.

2. The peptides according to claim 1, wherein the amino acid in P1 position is a cysteine, an analog or a derivative thereof.

3. The peptides according to claim 2, wherein the amino acid in P1 position is selected from the group comprising L-cysteine, D-cysteine, homocysteine, S-methylcysteine, alanine, S-ethylcysteine, threonine, methionine, serine and penicillamine.

4. The peptides according to any of claims 1 to 3, having in P6 position an acid function.

5. The peptides according to claim 4, said acid function in P6 position being selected from the group comprising aspartic acid, succinic acid and acylsulfonamide.

6. The peptides according to any of claims 1 to 5, having in P5 position an acid function.

7. The peptides according to claim 6, said acid function in P5 position being selected from the group comprising aspartic acid, succinic acid, acylsulfonamide.

8. The peptides according to any of claims 1 to 7, having in the P4 position an hydrophobic amino acid.

9. The peptides according to claim 8, said amino acid in P4 position being selected from the group comprising 3,3,diphenilalanine, leucine, isoleucine and phenylglicine.

10. The peptides according to any of claims 1 to 9, having in the position P3 an amino acid selected from the group comprising glutamic acid, valine and

isoleucine.

11. The peptides according to claim 10, having in the position P5 an amino acid selected from the group comprising aspartic acid, p-nitrophenylalanine, tyrosine, g-carboxyglutamic acid, D-phenylalanine, D-tyrosine, D-valine, D-isoleucine, D-3,3-diphenylalanine, D-aspartic acid, D-glutamic acid and D-g-carboxyglutamic acid.

12. The peptides according to claim 10 or 11, having in the position P1 an amino acid selected from the group comprising aminobutyric acid, norvaline and valine.

13. The peptides according to any of claims 1 to 12, wherein said peptides are capable of inhibiting 50% of the NS3 enzymatic activity at a concentration lower than or equal to 2 μM (IC_{50}).

14. The peptides according to any of claims 1 to 13, having in the positions P4, P3, P2 and P1, the amino acids naturally occurring respectively in P4, P3, P2 and P1 positions of one of the junction sites of the HCV virus, said junction sites being selected from the group comprising NS3/NS4A, NS4A/NS4B, NS4B/NS5A, and NS5A/NS5B.

15. The peptides according to claim 14, said peptides being obtainable by the proteolysis reaction of polipeptides containing at least one of the junction sites of the polyprotein of said HCV virus, said junction sites being selected from the group consisting of NS3/NS4A, NS4A/NS4B, NS4B/NS5A and NS5A/NS5B junction sites.

16. The peptides according to claim 15, wherein said junction sites consist of decapeptides, containing the amino acids naturally occurring in the positions P4, P3, P2 and P1 of said junction sites.

17. The peptides according to any of claims 14 to 16, wherein said HCV virus is selected from the group comprising the HCV viruses of 1a, 1b, 1c, 2a, 2b, 2c,

2d, 2e, 2f, 3a, 3b, 3c, 3d, 3e, 3f, 4a, 4b, 4c, 4d, 5a, 5a, 6b, 7a, 7b, 7c, 7d, 8a, 8b, 9a, 9b, 9c, 10a and 11a genotype.

18. The peptides according to claim 17, wherein said HCV virus is selected from the group comprising the HCV viruses of H-FDA, H-AP, HCV-1, HCV-J, HCV-BK, HC-J6, HCV-T, HC-J8, HCV-JT and HCV-JT' strain.

19. Peptides having an amino acid sequence selected from the group comprising the sequences reported in the annexed sequence listing as from SEQ ID NO:1 to SEQ ID NO:69.

20. Use of the peptides according to any of the claims from 1 to 19, for the derivation of binding or inhibition assays of the enzymatic activity of the NS3 protease of the HCV virus

21. Use of the peptides according to any of the claims from 1 to 19, for the preparation of drugs for the treatment of the non-A non-B hepatitis.

22. Pharmaceutical compositions for the treatment of the non-A non-B hepatitis, characterized in that they comprise at least one peptide according to any of claims 1 to 19 and a pharmaceutically effective carrier, vheicle or auxiliary agent.

23. Compositions for inhibiting the protease activity of the HCV virus associated to the NS3 protein, characterized in that they comprise at least one peptide according to any of claims 1 to 19.

24. A process for the production of at least a peptide according to any one of claims 1 to 19 characterized by the step of carrying out the the proteolysis of polypeptides containing at least one among the sequences of the NS3/NS4A, NS4A/NS4B, NS4B/NS5A and/or NS5A/NS5B junction sites of the polyprotein of HCV virus.

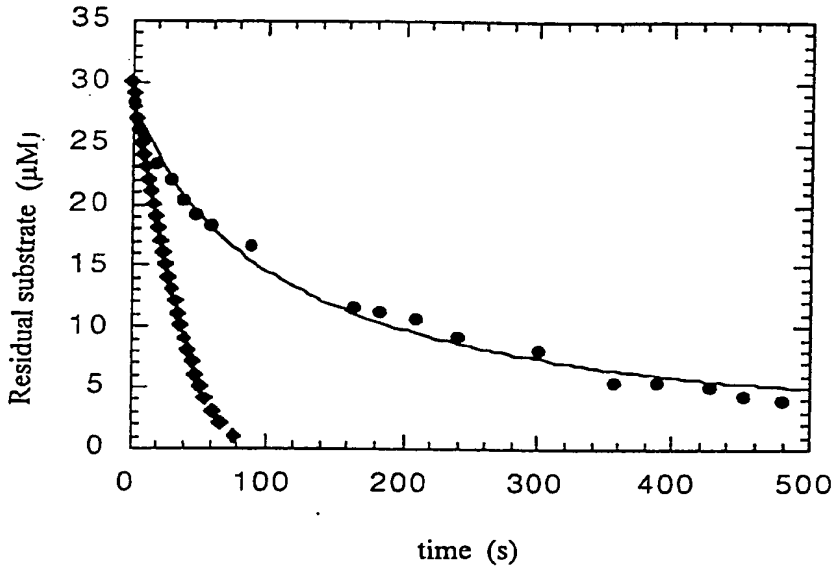
25. The process according to claim 24, wherein the proteolysis reaction is operated by NS3 protease of the HCV virus.

26. The process according to claim 24 or 25, wherein the HCV. virus is selected from the group comprising the HCV viruses of 1a, 1b, 1c, 2a, 2b, 2c, 2d, 2e, 2f, 3a, 3b, 3c, 3d, 3e, 3f, 4a, 4b, 4c, 4d, 5a, 5a, 6b, 7a, 7b, 7c, 7d, 8a, 8b, 9a, 9b, 9c, 10a and 11a genotype.

27. The process according to claim 26, wherein the HCV virus is selected from the group comprising the HCV viruses of H-FDA, H-AP, HCV-1, HCV-J, HCV-BK, HC-J6, HCV-T, HC-J8, HCV-JT and HCV-JT' strain.

28. The process according to any one of the claims from 24 to 27, wherein the junction sites consist of decapeptides, containing the amino acids naturally occurring in the positions P4, P3, P2 and P1 of said junction sites.

CLEAVAGE OF THE SUBSTRATE 4A/4B
CATALYZED BY NS3 PROTEASE



- experimental points
- ◆ theoretical points calculated in absence of inhibition by the product

Fig. 1

DETERMINATION OF THE IC50 OF
PEPTIDE SEQ. ID. No. 1

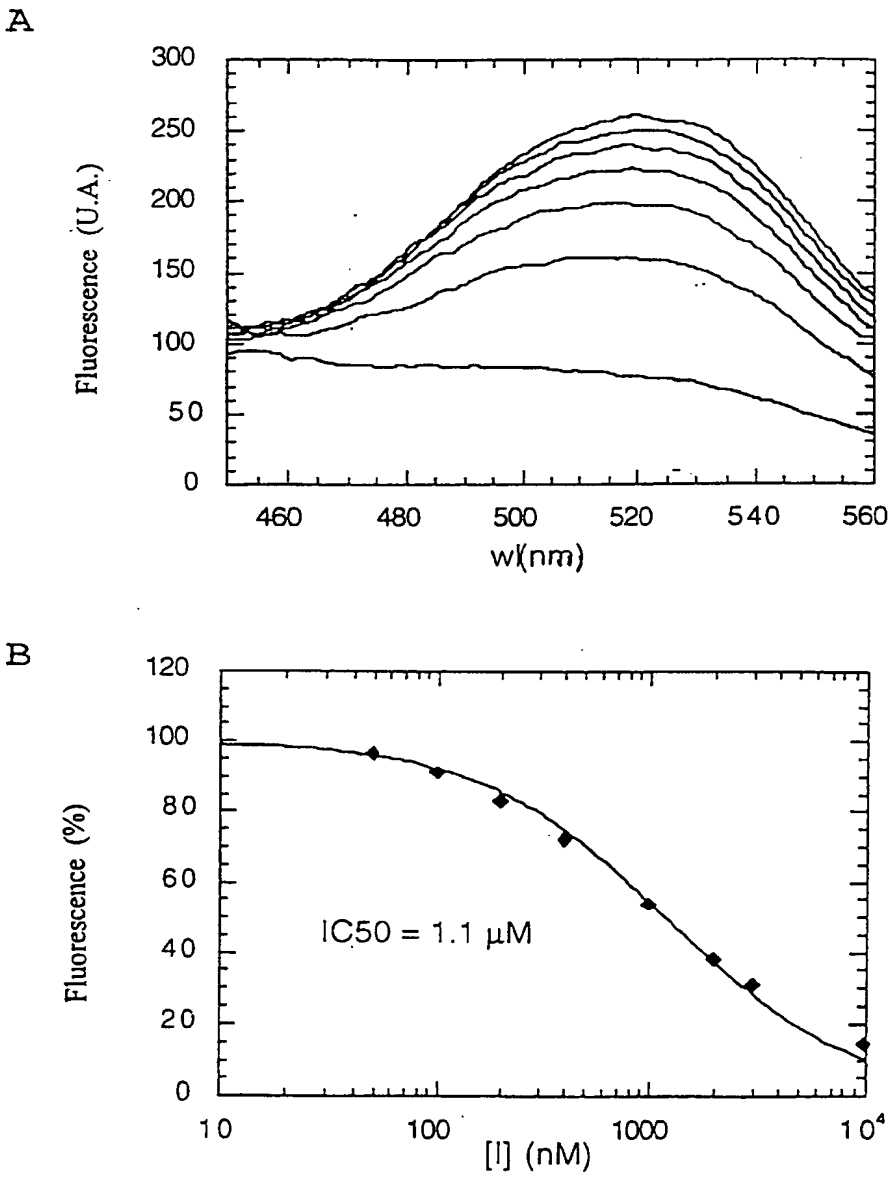


Fig. 2

SEQUENCE LISTING

GENERAL INFORMATIONS:

(i) APPLICANT: ISTITUTO DI RICERCHE DI BIOLOGIA MOLECOLARE P. ANGELETTI S.p.A.

(ii) TITLE OF INVENTION: PEPTIDES INHIBITORS OF THE SERINE PROTEASE ACTIVITY ASSOCIATED TO NS3 PROTEIN OF HCV, RELEVANT USES AND PROCESS OF PRODUCTION.

(iii) NUMBER OF SEQUENCES: 69

(iv) MAILING ADDRESS:

(A) ADDRESSEE: Società Italiana Brevetti

(B) STREET: Piazza di Pietra, 39

(C) CITY: Roma

(D) COUNTRY: Italia

(E) POST CODE: I-00186

(v) COMPUTER-READABLE FORM:

(A) TYPE OF SUPPORT:

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS-DOS Rev. 5.0

(D) SOFTWARE: Microsoft Word 6.0

(viii) AGENT INFORMATION

(A) NAME: DI CERBO, Mario (Dott.)

(B) REFERENCE: RM/X89216/PC-DC-EBR

(ix) TELECOMMUNICATIONS INFORMATION

(A) TELEPHONE: 06/695441

(B) TELEFAX: 06/69544830

(C) TELEX: 612287 ROPAT

(1) INFORMATION ON SEQUENCE SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 1:

Asp Glu Met Glu Glu Cys

1 5

(2) INFORMATION ON SEQUENCE SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 6

(D) OTHER INFORMATION: Xaa is D-cysteine

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 2:

Asp Glu Met Glu Glu Xaa
1 5

(3) INFORMATION ON SEQUENCE SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 6

(D) OTHER INFORMATION: Xaa is Abu

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 3:

Asp Glu Met Glu Glu Xaa
1 5

(4) INFORMATION ON SEQUENCE SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 4:

Asp Glu Met Glu Glu Ser

1 5

(5) INFORMATION ON SEQUENCE SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 5:

Asp Glu Met Glu Glu Gly

1 5

(6) INFORMATION ON SEQUENCE SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 4 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 6:

Met Glu Glu Cys

1

(7) INFORMATION ON SEQUENCE SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 5 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 7:

Glu Met Glu Glu Cys

1 5

(8) INFORMATION ON SEQUENCE SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 8:

Glu Asp Val Val Cys Cys
1 5

(9) INFORMATION ON SEQUENCE SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 5

(D) OTHER INFORMATION: Xaa is Abu

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 9:

Glu Asp Val Val Xaa Cys
1 5

(10) INFORMATION ON SEQUENCE SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 10:

Asp Glu Val Val Cys Cys

1 5

(11) INFORMATION ON SEQUENCE SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 11:

Glu Asp Val Val Gly Cys

1 5

(12) INFORMATION ON SEQUENCE SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 6

(D) OTHER INFORMATION: Xaa is Allyl-glycine

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 12:

Asp Glu Met Glu Glu Xaa

1 5

(13) INFORMATION ON SEQUENCE SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 5

(D) OTHER INFORMATION: Xaa is MeGly

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 13:

Glu Asp Val Val Xaa Cys

1 5

(14) INFORMATION ON SEQUENCE SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 6

(D) OTHER INFORMATION: Xaa is cysteamine

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 14:

Asp Glu Met Glu Glu Xaa

1 5

(15) INFORMATION ON SEQUENCE SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 4

(D) OTHER INFORMATION: Xaa is MeVal

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 5

(D) OTHER INFORMATION: Xaa is Abu

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 15:

Glu Asp Val Xaa Xaa Cys

1 5

(16) INFORMATION ON SEQUENCE SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 3

(D) OTHER INFORMATION: Xaa is MeVal

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 5

(D) OTHER INFORMATION: Xaa is Abu

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 16:

Glu Asp Xaa Val Xaa Cys

1 5

(17) INFORMATION ON SEQUENCE SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 6

(D) OTHER INFORMATION: Xaa is Cys-SOH

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 17:

Asp Glu Met Glu Glu Xaa

1 5

(18) INFORMATION ON SEQUENCE SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 5 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 1

(D) OTHER INFORMATION: Xaa is Glu whereto a succinyl group is bound

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 18:

Xaa Met Glu Glu Cys

1 5

(19) INFORMATION ON SEQUENCE SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 4 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 1

(D) OTHER INFORMATION: Xaa is Met whereto a succinil group is bound

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 19:

Xaa Glu Glu Cys

1 4

(20) INFORMATION ON SEQUENCE SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 5 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 1

(D) OTHER INFORMATION: Xaa is Glu displaying an Acylsulfonamide in N-terminal position

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 20:

Xaa Met Glu Glu Cys

1 5

(21) INFORMATION ON SEQUENCE SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(B) POSITION: 6

(D) OTHER INFORMATION: Xaa is Vinylglycine

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 21:

Asp Glu Met Glu Glu Xaa

1 5

(22) INFORMATION ON SEQUENCE SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 22:

Asp Glu Met Glu Leu Cys

1 5

(23) INFORMATION ON SEQUENCE SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 5

(D) OTHER INFORMATION: Xaa is β-cyclohexylalanine

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 23:

Asp Glu Met Glu Xaa Cys

1 5

(24) INFORMATION ON SEQUENCE SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 5

(D) OTHER INFORMATION: Xaa is naphtylalanine

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 24:

Asp Glu Met Glu Xaa Cys

1 5

(25) INFORMATION ON SEQUENCE SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 5 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 1

(D) OTHER INFORMATION: Xaa is Asp whereto a succinyl group is bound

(ix) FEATURE

(A) NAME: PEPTIDE

(B) POSITION: 5

(D) OTHER INFORMATION: Xaa is Abu

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 25:

Xaa Val Val Xaa Cys

1 5

(26) INFORMATION ON SEQUENCE SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 5

(D) OTHER INFORMATION: Xaa is Abu

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 6

(D) OTHER INFORMATION: Xaa is D-cysteine

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 26:

Glu Asp Val Val Xaa Xaa

1 5

(27) INFORMATION ON SEQUENCE SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 5

(D) OTHER INFORMATION: Xaa is S-methylcystein

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 27:

Asp Glu Met Glu Glu Xaa

1 5

(28) INFORMATION ON SEQUENCE SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 5

(D) OTHER INFORMATION: Xaa is β-cyclohexylalanine

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 28:

Asp Glu Val Glu Xaa Cys

1 5

(29) INFORMATION ON SEQUENCE SEQ ID NO: 29:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 5

(D) OTHER INFORMATION: Xaa is β-cyclohexylalanine

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 29:

Asp Glu Ile Glu Xaa Cys

1 5

(30) INFORMATION ON SEQUENCE SEQ ID NO: 30:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 5

(D) OTHER INFORMATION: Xaa is β-cyclohexylalanine

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 30:

Asp Glu Tyr Glu Xaa Cys
1 5

(31) INFORMATION ON SEQUENCE SEQ ID NO: 31:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 5

(D) OTHER INFORMATION: Xaa is β-cyclohexylalanine

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 31:

Asp Glu Phe Glu Xaa Cys
1 5

(32) INFORMATION ON SEQUENCE SEQ ID NO: 32:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 5

(D) OTHER INFORMATION: Xaa is β-cyclohexylalanine

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 32:

Asp Glu Leu Glu Xaa Cys

1 5

(33) INFORMATION ON SEQUENCE SEQ ID NO: 33:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 3

(D) OTHER INFORMATION: Xaa is β-cyclohexylalanine

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 5

(D) OTHER INFORMATION: Xaa is β-cyclohexylalanine

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 33:

Asp Glu Xaa Glu Xaa Cys

1 5

(34) INFORMATION ON SEQUENCE SEQ ID NO: 34:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 3

(D) OTHER INFORMATION: Xaa is Nle

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 5

(D) OTHER INFORMATION: Xaa is β-cyclohexylalanine

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 34:

Asp Glu Xaa Glu Xaa Cys

1 5

(35) INFORMATION ON SEQUENCE SEQ ID NO: 35:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 3

(D) OTHER INFORMATION: Xaa is 3,3-diphenylalanine

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 5

(D) OTHER INFORMATION: Xaa is β-cyclohexylalanine

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 35:

Asp Glu Xaa Glu Xaa Cys

1 5

(36) INFORMATION ON SEQUENCE SEQ ID NO: 36:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 3

(D) OTHER INFORMATION: Xaa is 2-tienylalanine

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 5

(D) OTHER INFORMATION: Xaa is β-cyclohexylalanine

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 36:

Asp Glu Xaa Glu Xaa Cys

1 5

(37) INFORMATION ON SEQUENCE SEQ ID NO: 37:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 3

(D) OTHER INFORMATION: Xaa is 4-chlorophenylalanine

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 5

(D) OTHER INFORMATION: Xaa is β-cyclohexylalanine

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 37:

Asp Glu Xaa Glu Xaa Cys

1 5

(38) INFORMATION ON SEQUENCE SEQ ID NO: 38:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 3

(D) OTHER INFORMATION: Xaa is phenylglycine

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 5

(D) OTHER INFORMATION: Xaa is β-cyclohexylalanine

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 38:

Asp Glu Xaa Glu Xaa Cys

1 5

(39) INFORMATION ON SEQUENCE SEQ ID NO: 39:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 3

(D) OTHER INFORMATION: Xaa is 3,3 diphenylalanine

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 5

(D) OTHER INFORMATION: Xaa is β-cyclohexylalanine

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 6

(D) OTHER INFORMATION: Xaa is D-cysteine

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 39:

Asp Glu Xaa Glu Xaa Xaa
1 5

(40) INFORMATION ON SEQUENCE SEQ ID NO: 40:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 6

(D) OTHER INFORMATION: Xaa is bAla

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 40:

Asp Glu Met Glu Glu Xaa
1 5

(41) INFORMATION ON SEQUENCE SEQ ID NO: 41:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 5

(D) OTHER INFORMATION: Xaa is Cys displaying an acylsulfonamide in C-terminal position

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 41:

Asp Glu Met Glu Glu Xaa

1 5

(42) INFORMATION ON SEQUENCE SEQ ID NO: 42:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 5 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 2

(D) OTHER INFORMATION: Xaa is 3,3 diphenylalanine

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 4

(D) OTHER INFORMATION: Xaa is β-cyclohexylalanine

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 42:

Glu Xaa Glu Xaa Cys

1 5

(43) INFORMATION ON SEQUENCE SEQ ID NO: 43:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 4 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 1

(D) OTHER INFORMATION: Xaa is 3,3 diphenylalanine

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 3

(D) OTHER INFORMATION: Xaa is β-cyclohexylalanine

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 43:

Xaa Glu Xaa Cys

1

(44) INFORMATION ON SEQUENCE SEQ ID NO: 44:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 5

(D) OTHER INFORMATION: Xaa is β-cyclohexylalanine

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 44:

Asp Glu Leu Val Xaa Cys

1 5

(45) INFORMATION ON SEQUENCE SEQ ID NO: 45:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 5

(D) OTHER INFORMATION: Xaa is β-cyclohexylalanine

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 45:

Asp Glu Leu Ile Xaa Cys
1 5

(46) INFORMATION ON SEQUENCE SEQ ID NO: 46:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 2

(D) OTHER INFORMATION: Xaa is methyl-glutamic acid

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 5

(D) OTHER INFORMATION: Xaa is β-cyclohexylalanine

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 46:

Asp Xaa Leu Glu Xaa Cys
1 5

(47) INFORMATION ON SEQUENCE SEQ ID NO: 47:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 3

(D) OTHER INFORMATION: Xaa is 3,3 diphenylalanine

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 5

(D) OTHER INFORMATION: Xaa is β-cyclohexylalanine

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 6

(D) OTHER INFORMATION: Xaa is dehydroalanine

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 47:

Asp Glu Xaa Glu Xaa Xaa

1 5

(48) INFORMATION ON SEQUENCE SEQ ID NO: 48:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 6

(D) OTHER INFORMATION: Xaa is 1-amino-1-ciclopentan-carboxylic acid

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 48:

Asp Glu Met Glu Glu Xaa

1 5

(49) INFORMATION ON SEQUENCE SEQ ID NO: 49:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 5 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 3

(D) OTHER INFORMATION: Xaa is 3,3 diphenylalanine

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 6

(D) OTHER INFORMATION: Xaa is β-cyclohexylalanine

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 49:

Asp Glu Xaa Ile Xaa

1 5

(50) INFORMATION ON SEQUENCE SEQ ID NO: 50:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 5 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 2

(D) OTHER INFORMATION: Xaa is 3,3 diphenylalanine

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 4

(D) OTHER INFORMATION: Xaa is β-cyclohexylalanine

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 50:

Glu Xaa Ile Xaa Cys

1 5

(51) INFORMATION ON SEQUENCE SEQ ID NO: 51:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 4 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 1

(D) OTHER INFORMATION: Xaa is 3,3 diphenylalanine

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 4

(D) OTHER INFORMATION: Xaa is β-cyclohexylalanine

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 51:

Xaa Ile Xaa Cys

1

(52) INFORMATION ON SEQUENCE SEQ ID NO: 52:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 6

(D) OTHER INFORMATION: Xaa is cyanoalanine

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 52:

Asp Glu Met Glu Glu Xaa

1 5

(53) INFORMATION ON SEQUENCE SEQ ID NO: 53:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 3

(D) OTHER INFORMATION: Xaa is 3,3 diphenylalanine

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 5

(D) OTHER INFORMATION: Xaa is β-cyclohexylalanine

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 53:

Asp Glu Xaa Ile Xaa Cys

1 5

(54) INFORMATION ON SEQUENCE SEQ ID NO: 54:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 5

(D) OTHER INFORMATION: Xaa is β-cyclohexylalanine

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 6

(D) OTHER INFORMATION: Xaa is Abu

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 54:

Asp Glu Leu Glu Xaa Xaa

1 5

(55) INFORMATION ON SEQUENCE SEQ ID NO: 55:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 5

(D) OTHER INFORMATION: Xaa is β-cyclohexylalanine

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 55:

Asp Glu Leu Glu Xaa Val

1 5

(56) INFORMATION ON SEQUENCE SEQ ID NO: 56:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 5

(D) OTHER INFORMATION: Xaa is β-cyclohexylalanine

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 6

(D) OTHER INFORMATION: Xaa is Nva

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 56:

Asp Glu Leu Glu Xaa Xaa

1 5

(57) INFORMATION ON SEQUENCE SEQ ID NO: 57:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 5

(D) OTHER INFORMATION: Xaa is β-cyclohexylalanine

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 57:

Asp Asp Leu Glu Xaa Cys

1 5

(58) INFORMATION ON SEQUENCE SEQ ID NO: 58:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 2

(D) OTHER INFORMATION: Xaa is 4-nitrophenylalanine

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 5

(D) OTHER INFORMATION: Xaa is β-cyclohexylalanine

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 58:

Asp Xaa Leu Glu Xaa Cys

1 5

(59) INFORMATION ON SEQUENCE SEQ ID NO: 59:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 5

(D) OTHER INFORMATION: Xaa is β-cyclohexylalanine

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 59:

Asp Tyr Leu Glu Xaa Cys

1 5

(60) INFORMATION ON SEQUENCE SEQ ID NO: 60:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 2

(D) OTHER INFORMATION: Xaa is g-carboxyglutamic acid

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 5

(D) OTHER INFORMATION: Xaa is β-cyclohexylalanine

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 60:

Asp Xaa Leu Glu Xaa Cys

1 5

(61) INFORMATION ON SEQUENCE SEQ ID NO: 61:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 2

(D) OTHER INFORMATION: Xaa is D-phenylalanine

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 5

(D) OTHER INFORMATION: Xaa is β-cyclohexylalanine

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 61:

Asp Xaa Leu Glu Xaa Cys

1 5

(62) INFORMATION ON SEQUENCE SEQ ID NO: 62:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 2

(D) OTHER INFORMATION: Xaa is D-tyrosine

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 5

(D) OTHER INFORMATION: Xaa is β-cyclohexylalanine

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 62:

Asp Xaa Leu Glu Xaa Cys

1 5

(63) INFORMATION ON SEQUENCE SEQ ID NO: 63:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 2

(D) OTHER INFORMATION: Xaa is D-valine

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 5

(D) OTHER INFORMATION: Xaa is β-cyclohexylalanine

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 63:

Asp Xaa Leu Glu Xaa Cys

1 5

(64) INFORMATION ON SEQUENCE SEQ ID NO: 64:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 2

(D) OTHER INFORMATION: Xaa is D-isoleucine

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 5

(D) OTHER INFORMATION: Xaa is β-cyclohexylalanine

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 64:

Asp Xaa Leu Glu Xaa Cys

1 5

(65) INFORMATION ON SEQUENCE SEQ ID NO: 65:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 2

(D) OTHER INFORMATION: Xaa is D-3,3 diphenylalanine

(ix) FEATURE

(A) NAME: PEPTIDE

(B) POSITION: 5

(D) OTHER INFORMATION: Xaa is β-cyclohexylalanine

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 65:

Asp Xaa Leu Glu Xaa Cys
1 5

(66) INFORMATION ON SEQUENCE SEQ ID NO: 66:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 2

(D) OTHER INFORMATION: Xaa is D-aspartic acid

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 5

(D) OTHER INFORMATION: Xaa is β-cyclohexylalanine

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 66:

Asp Xaa Leu Glu Xaa Cys
1 5

(67) INFORMATION ON SEQUENCE SEQ ID NO: 67:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 2

(D) OTHER INFORMATION: Xaa is D-glutamic acid

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 5

(D) OTHER INFORMATION: Xaa is β-cyclohexylalanine

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 67:

Asp Xaa Leu Glu Xaa Cys

1 5

(68) INFORMATION ON SEQUENCE SEQ ID NO: 68:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 2

(D) OTHER INFORMATION: Xaa is D-g-carboxyglutamic acid

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 5

(D) OTHER INFORMATION: Xaa is β-cyclohexylalanine

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 68:

Asp Xaa Leu Ile Xaa Cys

1 5

(69) INFORMATION ON SEQUENCE SEQ ID NO: 69:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 3

(D) OTHER INFORMATION: Xaa is β diamino propionic (N-b-dansyl) acid

(ix) FEATURE

(A) NAME: PEPTIDE

(B) LOCATION: 5

(D) OTHER INFORMATION: Xaa is β-cyclohexylalanine

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 69:

Asp Glu Xaa Glu Xaa Cys

1 5